Caryopsis Structural and Imbibitional Characteristics of Some Hard Red and White Wheats[1]

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ABSTRACT

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Caryopsis structure and water imbibition of red and white wheat cultivars were investigated to determine differences that might relate to sprouting characteristics of the two classes. Separation of integuments and seed coats of 14 cultivars was rated by scanning electron microscopy. Cultivars that differed in sprouting resistance were examined by scanning electron microscopy and light microscopy. Water imbibition of cultivars and penetration of ^3H_2O during germination were determined for cultivars that differed most in structural characteristics. White wheat cultivars exhibited looser integument structure and greater separation between the seed coat and tube cells of the inner pericarp than did red wheat cultivars. The pericarp of white wheats frequently was folded and generally was weaker than that of red wheats. Water was imbibed faster and penetrated deeper into the kernels of the white wheats than into kernels of the red wheats. Although caryopsis structural features of white wheats enhanced permeation of water and decreased mechanical impedance to embryo expansion during germination, they must be considered among the multiplicity of physiological factors that determine sprouting characteristics of wheat.

Dormancy of wheat (*Triticum aestivum* L.) is important in preharvest sprouting resistance and in the ability of seed to germinate when planting closely follows harvest (Belderok 1968). Lack of dormancy results in preharvest sprouting under favorable climatic conditions, which adversely affects grain functional qualities; conversely, excessive dormancy results in poor plant stands.

Differential dormancy and susceptibility to preharvest sprouting frequently are attributed to quantitative differences in chemical inhibitors in the integuments (Miyamoto et al 1961). The relationship is advanced to explain differences between red cultivars and white cultivars—which are presumed to lack the inhibitors—as well as differences among red cultivars themselves.

Few investigations have concerned the role of seed structural characteristics in sprouting resistance of wheat. An early study by Krauss (1933) showed that structural features of cells in the area of the micropyle differed between red and white wheats; he suggested that this difference might relate to control of water movement by the micropyle and thus to differences in sprouting resistance. Wellington (1956) concluded that mechanical impedance by the epidermis, not pigmentation or exclusion of water or oxygen, hindered germination of red wheats but not white wheats. Microscopic examination of ripening kernels showed that the testa layer of sprouting-susceptible red and white wheats separated into granular components, whereas the testa layer of sprouting-resistant red wheats was homogeneous (Belderok 1976).

The lack of information on ultrastructural and imbibitional characteristics of red and white wheats prompted investigation of cultivars previously shown to differ significantly in susceptibility to sprouting (Huang and Varriano-Marston 1980; McCrate et al 1981, 1982).

MATERIALS AND METHODS

Caryopsis Structure

Seed integument structure of 14 wheat cultivars grown in the greenhouse was examined with an ETEC U-1 scanning electron microscope operated at 20 kV. Kernels were fractured with a dull razor blade, mounted on aluminum stubs, and coated with carbon and gold-palladium. Preliminary examination revealed that tenacity of adherence of integuments to the seed coat differed substantially among cultivars. A rating scale of 0–4, based on the degree of separation between integuments and seed coats of different samples, was assigned to a series of photomicrographs. Rating of additional samples was determined on four observations per kernel on five kernels of each cultivar directly from the scanning electron microscope (SEM) screen, using the photomicrographs as a reference. Two replications of each cultivar were rated.

Two hard white winter wheats (Clark's Cream and KS73256) and one hard red winter wheat (Eagle) grown in the field were examined by light microscopy and scanning electron microscopy. Clark's Cream and Eagle are resistant to sprouting; KS73256 is susceptible. Growing conditions and sprouting resistance of the cultivars were reported previously (Huang and Varriano-Marston 1980). Samples of sound wheat were soaked in distilled water for 3 hr, frozen on a sample stub, and sectioned (12 μm) at −25°C on an American Optical cryotome. Sections were collected on glass slides and stained with 0.1% Ponceau 2R. Photomicrographs were taken on a Reichert (Austria) light microscope. Samples for scanning electron microscopy were prepared as described.

Water Imbibition

Water imbibition by kernels of four cultivars among the 14 tested that differed significantly in the rating of integument adherence was determined. One hundred kernel subsamples were placed on moistened filter paper sheets, covered with filter paper disks, and allowed to imbibe at 25°C in a 100% relative humidity chamber. Subsamples were removed at 4-hr intervals over a 24-hr period, blotted free of excess water, and weighed. They were then dried to a constant weight of 80°C and reweighed; water uptake was expressed as a percentage of dry weight. Samples were replicated four times. Water absorption of the three field-grown cultivars similarly was determined by immersing sound kernels for 1, 2, 3, or 6 hr at 25°C. Results also were expressed as a percentage of kernel dry weight.

Autoradiography

Thirty sound kernels were placed in 2-dram glass vials, and 0.2 ml of ^3H_2O (50 mCi/ml) was added to each vial from a syringe. The vials were shaken, held at 25°C for 1 or 3 hr, and the wheat kernels were prepared for autoradiography as described by Jackson and Varriano-Marston (1980).

RESULTS

Caryopsis Structure

The characteristic separation of the integuments and seed coat noted in wheat and examples of the 0–4 scale used to rate the separation are shown in Fig. 1. Separation occurred most frequently between the seed coat and tube cell layers of the inner pericarp, but separation was also occasionally observed between the inner and outer pericarp layers. Integument structure was significantly looser and varied more among white wheat cultivars

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than among red wheat cultivars (Table I). Red wheat cultivars did not differ significantly. Falcon and KS75216, two white wheats, had the greatest separation between integuments and seed coat. Two isogenic lines, KS74158 white and KS74158 red, were not significantly different, although the white line had a higher rating than the red line. KS77H2690, a white selection from Eagle, and KS75216, a white sister line of Newton, had significantly more separation of the integuments than their red counterparts.

Scanning electron micrographs of the outer layers of the three field-grown cultivars (Fig. 2) showed that the pericarp layer was densely packed. Folds in the pericarp of the white wheats (Fig. 2b, c), however, created air spaces within the pericarp.

Light microscope studies provided further evidence of differences in the covering layers of white- and red-grained wheats. During thin sectioning, the pericarp of the white wheats was damaged more easily than that of the red wheats (Fig. 3). In white wheat, the epidermis and hypodermis commonly were torn away from the other structures of the pericarp (Fig. 3b,c), suggesting that the pericarp was weaker in the white wheats than in the red wheats.

Differences in the hard and soft endosperms of the three cultivars were also noted. The KS73256 had a softer hard endosperm and a less compact soft endosperm (Fig. 4e,f) than did the two other cultivars. The hard endosperm of KS73256 more closely resembled the soft endosperm than the hard endosperm of the other cultivars.

Water Imbibition

KS75216 took up significantly more water at all sampling times during a 24-hr period than did Eagle, Newton, or KS77H2690 (data not shown). Newton, Eagle, and KS77H2690 did not differ significantly in water uptake at any sampling time.

The two field-grown white wheats imbibed more water than the red Eagle variety (Table II) after 1 hr. After 2, 3, and 6 hr, the amount of water absorbed by Eagle and Clark's Cream did not differ, but the white KS73256 cultivar imbibed significantly ($P < 0.05$) more water than the other cultivar.

Autoradiography

Considerable variability existed in the depth of water penetration into individual kernels. The autoradiographs presented in Figs. 5 and 6 represent the average variability observed. Water generally penetrated deeper into the kernels of the KS73256 wheat than the Eagle or Clark's Cream wheats after 1 and 3 hr. Therefore, not only did the kernels of the KS73256 imbibe more water (Table II), but the water penetrated deeper into the kernel structure than was the case for the other cultivars.

TABLE I
Mean Rating on 0 (Low) to 4 (High) Scale of Separation Between Integuments and Seed Coat of 14 Wheat Genotypes Determined with a Scanning Electron Microscope

Genotype	Class[a]	Separation (Rating)
Falcon	HWS	2.5
KS75216	HWW	2.2
Timstein	HWS	1.9
Burt	HWW	1.8
KS77H2690	HWW	1.7
Clark's Cream	HWW	1.7
Scout 66	HRW	1.4
KS74158-W	HWW	1.4
Centurk	HRW	1.3
Eagle	HRW	1.2
KS74158-R	HRW	1.2
Lindon	HRW	1.2
Newton	HRW	1.1
Parker 76	HRW	1.1
Mean		1.6
Least significant difference[b]		0.4

[a] HWS = Hard white spring, HWW = hard white winter, and HRW = hard red winter.
[b] $P = 0.05$.

DISCUSSION

The mode of germination inhibition of many structural features of the wheat caryopsis can be variously interpreted. Kernel covering layers, for instance, might exclude oxygen (Belderok 1968) or water (Krauss 1933), exert mechanical impedance (Wellington 1956), or contain chemical inhibitors (Miyamoto et al 1961). Any of those effects could be related to kernel color through

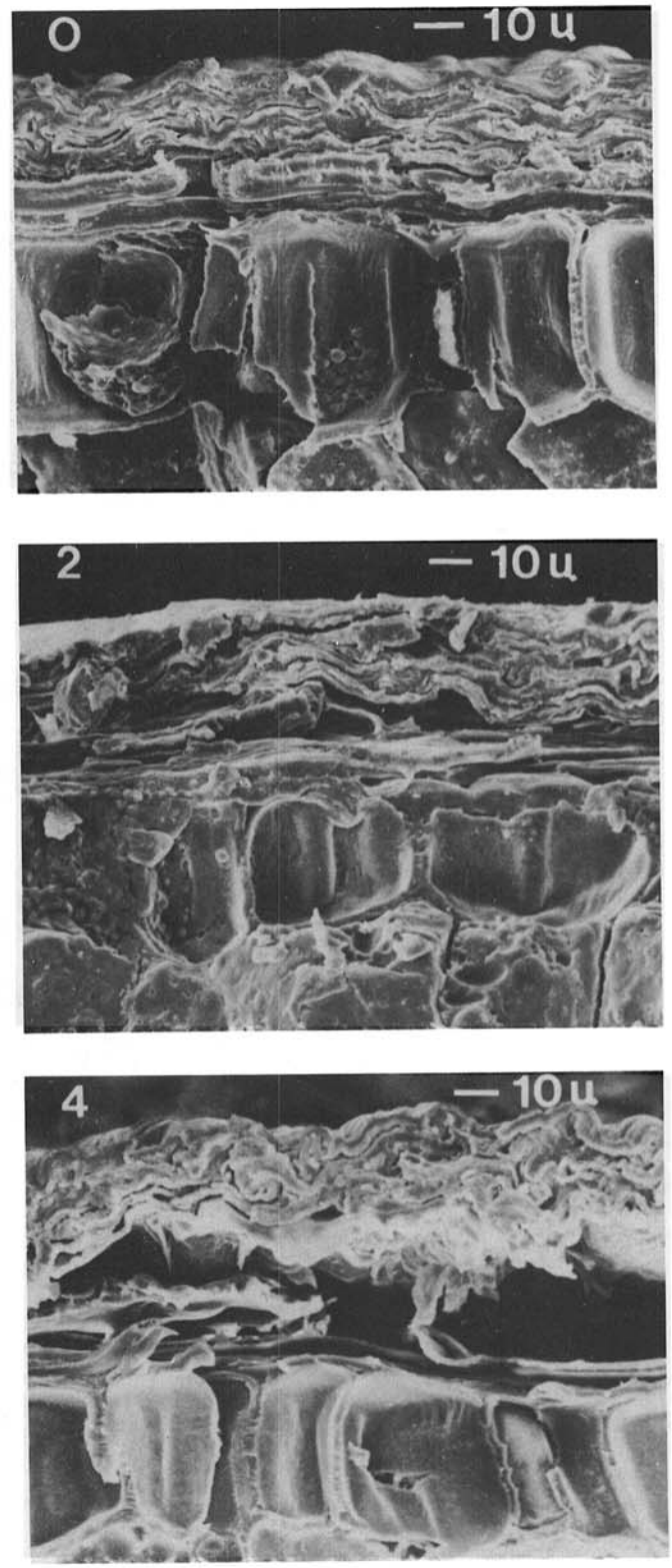

Fig. 1. Characteristic separation of integuments and seed coats of red and white wheat genotypes rated 0 (low), 2 (intermediate), and 4 (high) on a scale of 0–4.

gene pleiotropism or linkage (Belderok 1968). Moreover, phenolic compounds that give red wheats their distinctive color might hinder permeation of water as described for several *Pisum* species (Werker et al 1979).

Interpretation of structural differences between red and white wheats must distinguish between inherent differences and artifacts of incipient sprouting. Differences in integument structure, particularly between closely related lines, and the parallel differences in sprouting resistance of many of those lines (McCrate et al 1981a) suggested that structural differences are related to inherent sprouting susceptibility of the two wheat classes. The resemblance between some traits observed in the white wheats and early structural changes associated with sprouting (Swanson 1946, Wellington 1956) indicated, however, that differences should be

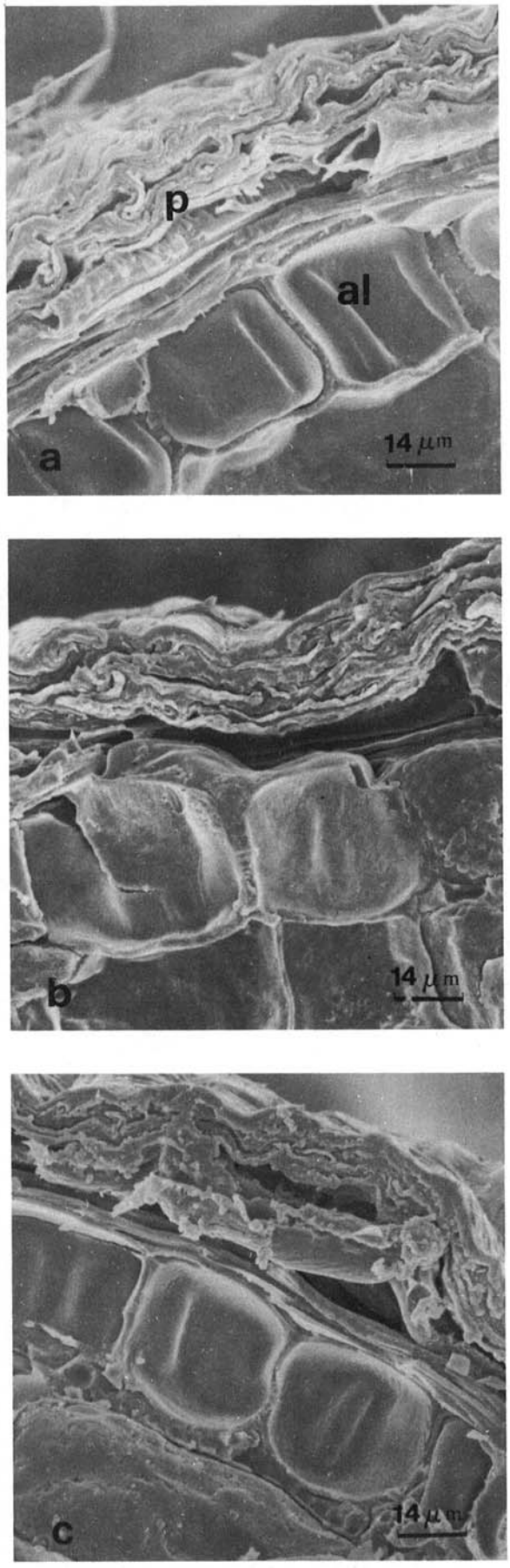

Fig. 2. Scanning electron micrographs of the covering layers of **a**, Eagle; **b**, Clark's Cream; and **c**, KS73256 wheat genotypes.

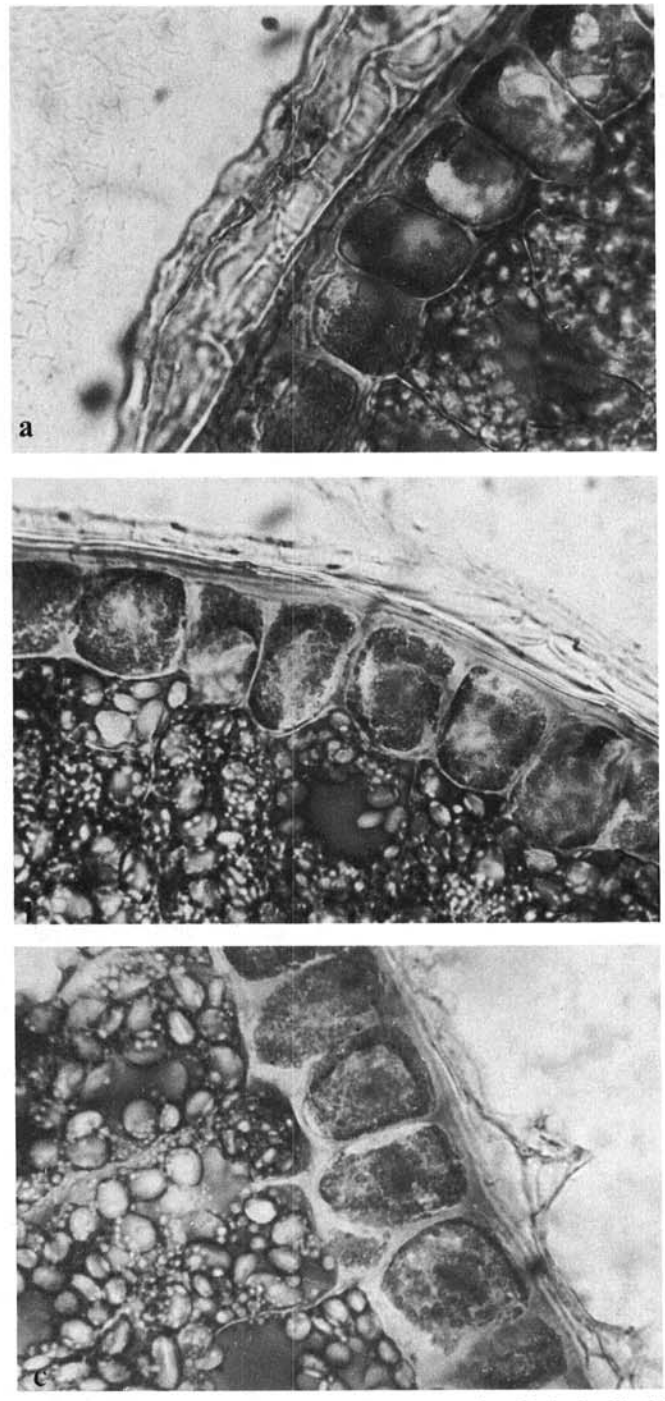

Fig. 3. Light micrographs of the covering layers of **a**, Eagle; **b**, Clark's Cream; and **c**, KS73256 wheat genotypes.

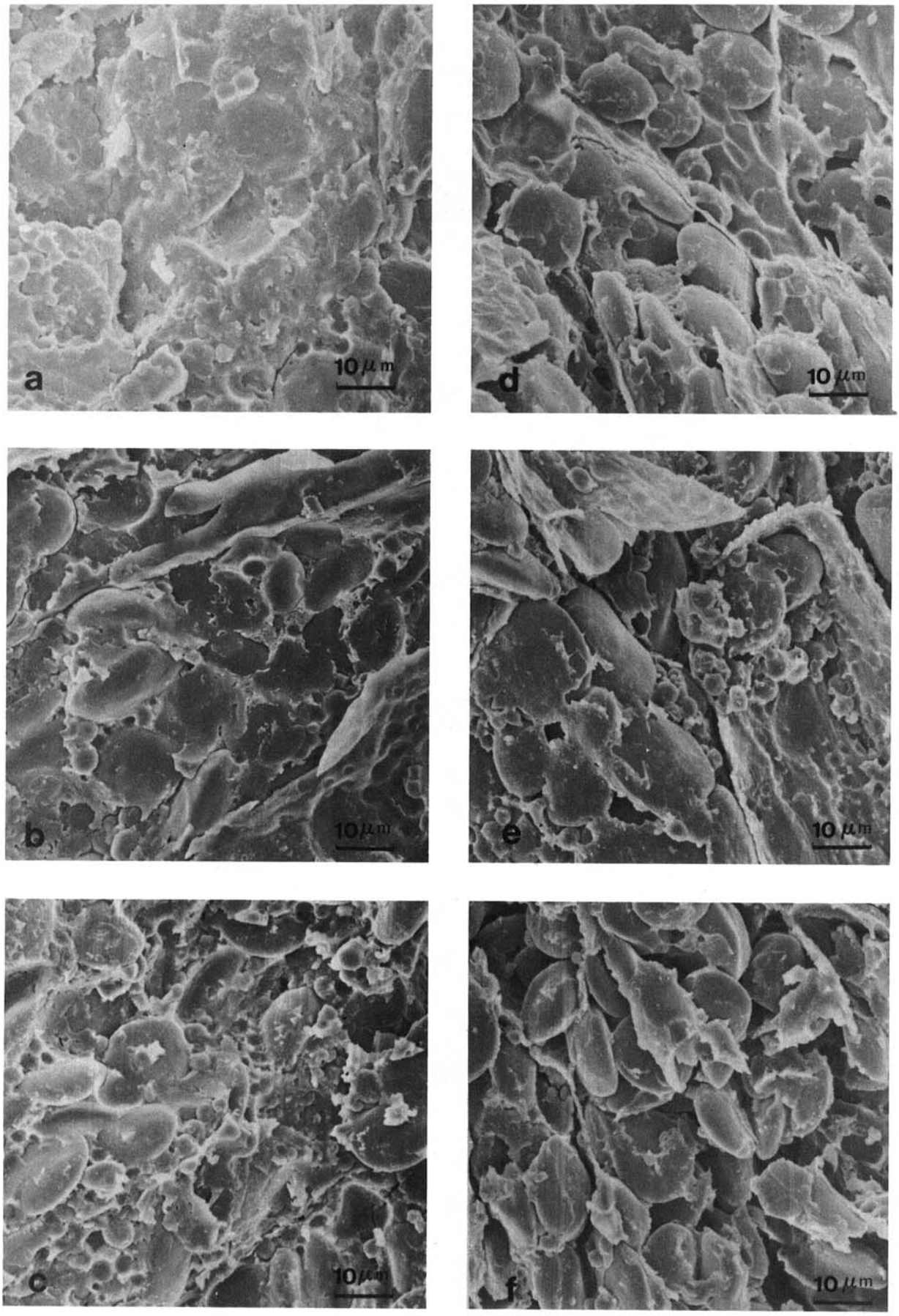

Fig. 4. Scanning electron micrographs of the hard (**a–c**) and soft (**d–f**) endosperms of Eagle (**a,d**), Clark's Cream (**b,e**), and KS73256 (**c,f**) wheat genotypes.

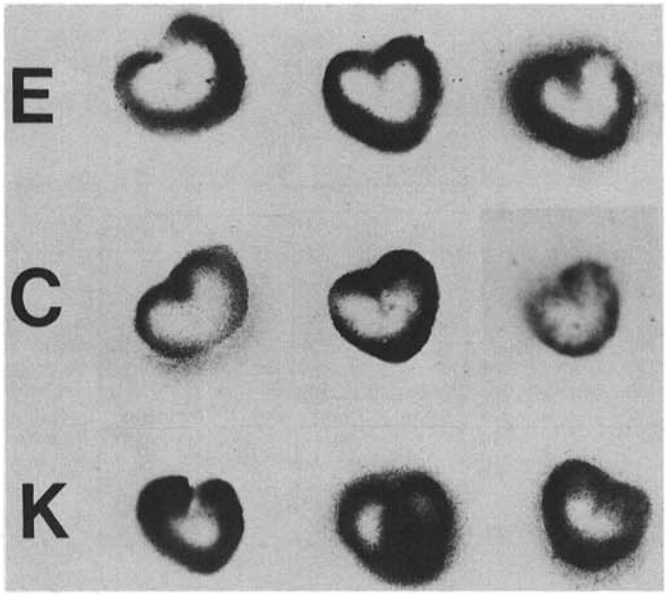

Fig. 5. Autoradiograms of wheat kernels after 1 hr of conditioning at 25° C. E = Eagle, C = Clark's Cream, and K = KS73256.

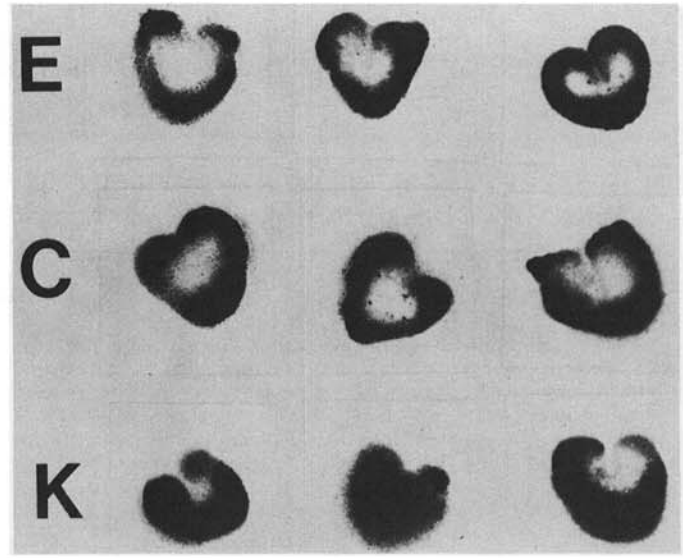

Fig. 6. Autoradiograms of wheat kernels after 3 hr of conditioning at 25° C. E = Eagle, C = Clark's Cream, and K = KS73256.

TABLE II
Water Imbibition by Kernels of Red and White Wheat Genotypes After Four Durations of Immersion at 25° C

	Water Imbibition[a] During Immersion for			
Genotype	**1 hr**	**2 hr**	**3 hr**	**6 hr**
Eagle	8.9	14.0	17.4	25.1
Clark's Cream	11.0	14.5	18.2	25.8
KS73256	11.3	16.9	21.2	32.6
Least significant difference[b]	0.8			

[a] Percent dry weight.
[b] $P = 0.05$.

carefully examined.

Differences in caryopsis structure are probably one of a multiplicity of factors that make white wheats more susceptible than red wheats to preharvest sprouting. Wellington (1956) concluded that neither water nor oxygen permeation differed between the two classes during early germination stages, but that mechanical impedance of the epidermis of red wheats to rupture during later stages was the critical difference. Structural differences observed by us might contribute to either effect. The rapidity with which water uptake and penetration differed—which was well before mechanical impedance occurs (Wellington 1956)—suggested that that may be critical. Some of the same structural features in white wheats that allow faster water permeation during early germination, however, would lessen impedance to embryo expansion during later stages.

The fact that caryopsis features are only partially involved in germination behavior of wheat was indicated by evidence (McCrate et al 1982, Stoy and Olsen 1980) that reaction of excised embryos to germination inhibitors paralleled sprouting resistance of the cultivars from which the embryos were isolated. Thus, neither caryopsis structure nor its color or composition is alone responsible for sprouting characteristics of wheat cultivars or classes.

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